attached antigen is important for B cell signaling. B cells gather antigen from a variety of sources which may have different physical characteristics such as mobility, stiffness or topography. However, the effect of these parameters on BCR clustering and signaling activation is not understood. We have studied the interaction of B cells with BCR ligand coated surfaces to investigate the physical parameters affecting BCR microcluster formation, cell spreading and signaling activation. Using high-resolution TIRF microscopy of live cells, we followed the movement and spatial organization of BCR clusters and the dynamics of actin as well as the associated signaling on surfaces with different physical properties. Using glass and lipid bilayer surfaces, we found that both immobile and mobile ligands are able to crosslink BCRs and induce clustering. However, B cells interacting with mobile ligands (on lipid bilayer) displayed greater signaling than those interacting with immobile ligands (on glass). Quantitative analysis revealed that mobile ligands enabled BCR clusters to move farther and merge more efficiently than immobile ligands. We also investigated the effect of substrate topography and substrate stiffness on BCR and actin dynamics. Quantitative analysis showed that these parameters were associated with differences in actin remodeling and spreading behavior. Our results indicate that B cells are highly sensitive to a range of physical parameters during cell spreading and signaling activation.

3962-Pos Board B690

The Actin Crosslinking Protein Palladin Modulates Force Generation and Mechanical Sensing of Tumor Associated Fibroblasts

Mikheil Azatov[1], Silvia Goicoechea[2], Carol Otey[2], Rosa Hwang[3], Arpita Upadhyaya[1].
[1]University of Maryland, College Park, MD, USA, [2]UNC School of Medicine, Chapel Hill, NC, USA, [3]University of Texas M.D.Anderson Cancer Center, Houston, TX, USA.

Cells organize actin filaments into higher-order structures by regulating the composition, distribution and concentration of actin crosslinkers. Palladin is an actin-crosslinking protein that is found in the lamellar actin network and stress fibers, two actin structures critical for mechanosensing of the physical environment. Palladin also serves as a molecular scaffold for alpha-actinin, a key actin crosslinker. By virtue of its close interactions with actomyosin structures in the cell, palladin may play an important role in cell mechanics. However, the role of palladin in cellular force generation and mechanosensing has not been studied. In this study we use human pancreatic tumor associated fibroblasts (TAFs) to investigate the role of palladin in regulating the plasticity of the actin cytoskeleton and cellular force generation in response to alterations in substrate stiffness. Traction force microscopy revealed that TAFs are sensitive to substrate stiffness as they generate larger forces on substrates of increased stiffness. Contrary to expectations, knocking down palladin increased the forces generated by cells, and also inhibited the ability to sense substrate stiffness for very stiff gels. This was accompanied by significant differences in the actin organization and adhesion dynamics of palladin knock down cells. Perturbation experiments also suggest altered myosin activity in palladin KD cells. Our results suggest that the actin crosslinkers such as palladin and myosin motors coordinate for optimal cell function and to prevent aberrant behavior as in cancer metastasis.

3963-Pos Board B691

Mechanical Stress in Actinin and Actin in Stem Cells

Fanjie Meng[1], Jun Guo[2], Frederick Sachs[1].
[1]University at Buffalo, Buffalo, NY, USA, [2]Nanjing Medical University, Nanjing, NY, USA.

cpstFRET is a FRET based force sensor designed to be modulated primarily by the angle between the donor and acceptor. It is physically smaller, less invasive, and has greater dynamic range and better signal-to-noise behavior than the linear probes. Using this sensor with FRET polarization imaging we measured the gradients of constitutional stresses in time and space for actinin and actin in HEK and MDCK cells. We created eight stable cell lines with these probes. Then we derived stem cells from the cell lines and measured stress changes during differentiation and dedifferentiation. We calculated FRET with the parallel/perpendicular polarization images of FRET. Because the FRET signal is always more depolarized, high ratios represent low FRET and high stress, while low ratios represent high FRET and low stress. We induced HEK and MDCK cells into embryoid bodies (EB) which is the characteristic morphology of induced pluripotent stem cells and cancer stem cells. We verified the stem cell in the EBs with alkaline phosphatase (AP) staining. Stem cells in EBs derived from HEKs showed higher AP activities than those derived from MDCK cells. All stem cells showed escalated stress in both actinin and actin relative to the parent. Stress was higher in stem cells of HEK origination. After we removed the EB-induction factors, these stresses declined as the stem cells differentiated into HEK or epithelial cells. We also induced the stem cells differentiate into neurons. The cell body showed low stress while axon extrusions showed increased stress in actin and actinin. The significant stress changes in stem cells and differentiated descendants hint at the potential of inducing pluripotent stem cells through changing cell mechanics. The data also shows that stem cell differentiation involves changes in internal stresses as well as changes in biochemistry.

3964-Pos Board B692

Single Molecule Mechano-Memory

Isaac T.S. Li[1], Taekjip Ha[1,2], Yann R. Chemla[1,3].
[1]Center for the Physics of Living Cells and Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Institute for Genomic Biology, Howard Hughes Medical Institute, and Center for Biophysics and Center, Urbana, IL, USA, [3]Center for Biophysics and Computational Biology, Urbana, IL, USA.

Understanding the spatial distribution of individual adhesion bonds and the tension exerted on them is crucial for understanding whole cell adhesion behavior. Here, we introduce a new class of molecular force sensors to record cellular adhesion events at the single-molecule level. A DNA structure was designed that responds to mechanical perturbation above certain threshold tension and maintains a memory of that perturbation. We name this feature "single-molecule mechano-memory" (smMM). The smMM sensor undergoes conformational changes under tension and is kinetically trapped under a new conformation. Single-molecule force spectroscopy and fluorescence spectroscopy were performed to characterize the activation force as well as memory life time. We show that in the absence of mechanical perturbation the smMM sensor is well folded and stable. In the presence of tension above ~35 pN, the sensor is converted to the unfolded "memory" state in which it remains kinetically trapped for an average of 25 seconds. Both activation force and life time of the sensor can be tuned by its DNA sequence. As a proof of concept for this class of sensors, smMM sensors were coated on a surface where cell adhesion takes place. Individual adhesion events are detected using fluorescently-labeled oligonucleotide probes to mark unfolded sensors.

3965-Pos Board B693

DNA-Based "Digital" Tension Probes with Pn Sensitivity Reveal Early Cell Adhesion Mechanics at the Single Molecule Level

Yun Zhang, Khalid Salaita.
Chemistry, Emory University, Atlanta, GA, USA.

Mechanical forces are involved in important processes such as cell division, migration and gene expression, signifying their critical importance to living systems. However, the lack of methods to visualize cellular forces at the molecular scale has hampered the study of mechanotransduction. To address this need, we developed a new class of DNA-based molecular tension fluorescence microscopy (MTFM) probes that function as a reversible digital switch, and are ideally suited to investigate the pN-range forces applied by individual integrins. We show that focal adhesion maturation involves an increase in tension per molecule coupled with recruitment of a greater density of integrins. By engaging cells to sensor chips presenting mixtures of spectrally-encoded probes with different mechanical responses, we find that integrins display both chemical and mechanical specificity at the single-molecule level. Integrins show mechanical preference for more rigid ligands within nascent adhesions, thus suggesting focal adhesions function as rigidity sensors at the single integrin level. Moreover, this observation may be related to the "catch" bond model, where the integrin-ligand bond lifetime increases under a mechanical load.

3966-Pos Board B694

Buckling of a Physically-Constrained Growing Epithelium

Anastasiya Trushko, Aurélien Roux.
University of Geneva, Geneva, Switzerland.

In growing tissue, cell proliferation and tensile/compressive forces generated by growth are essential for controlling shape and size of organs during development (Mammoto T and Ingber DE 2010). One example of a system where these parameters may have a critical influence is an early stage of embryogenesis, namely gastrulation. During gastrulation, the spherically shaped epithelium invaginates and forms a tube in the lumena of the embryo that later will be the digestive tube. The invagination of the epithelia cells is promoted by genetic factors. However, recent theoretical models proposed that compression of the embryo in a spherical shell could promote gastrulation by inward buckling (Hannezo E et al. 2011, Tamulonis C et al. 2011). As embryo is not easily amenable to forces and shape measurements, to study the coupling between mechanical forces/confinement and epithelium shape and proliferation, we create an epithelial spherical monolayer in the elastic shell using a technique of cell encapsulation in the shell made out of alginate. As the elastic modulus of alginate is known, small deformation of the shell will allow us to measure compression within the epithelium (Alessandri K et al. 2013). We then follow the growth of the epithelium through time and observe its buckling. We address the questions of how the pressure builds up in the growing epithelia enclosed in

a confined environment, what are the cell tension fields in the epithelial monolayer under these conditions, how the cells adapt to the pressure and the shape and rigidity of the confinement, and how the whole monolayer react to the accumulated lateral pressure due to cell proliferation. These will let me decipher how epithelium deformation is driven by cell proliferation and tension during development.

3967-Pos Board B695

Mitotic Cell Shape - RNA Interference Screening for Genes Involved in Mechanics using Atomic Force Microscopy

Cédric J. Cattin[1], Martin P. Stewart[1], Yusuke Toyoda[2], Ina Poser[2], Frank Buchholz[3], Anthony A. Hyman[2], Daniel J. Müller[1].
[1]ETH Zürich, Basel, Switzerland, [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, [3]UCC, Medical Systems Biology, Medical Faculty, Technical University Dresden, Dresden, Germany.

Cell division in animal cells requires major changes in the cytoskeletal arrangement to achieve accurate positioning of the mitotic spindle and subsequent correct chromosome segregation. To this end, cells undergo drastic shape changes in a process termed mitotic cell rounding. The mechanisms controlling this process are not fully understood. Using an atomic force microscopy (AFM)-based assay that quantifies cell mechanical parameters in conjunction with RNAi gene silencing, we screened almost 1000 genes for their role in mitotic cell mechanics. We find ~5 % of the genes screened to strongly influence mitotic cell mechanics and provide results from experiments further investigating the role of a subset of these genes formerly not known to be involved in this process. We believe that mechanical phenotyping like presented in this study can lead to new and quantitative insights into the way cells regulate and maintain their shape and mechanical integrity.

3968-Pos Board B696

Measuring Actomyosin Function in a Living Parasite using a Laser Trap

Rachel V. Stadler[1], Lauren A. White[1], Brian P. Helmke[1], Ke Hu[2], **William H. Guilford**[1].
[1]University of Virginia, Charlottesville, VA, USA, [2]Indiana University, Bloomington, Bloomington, IN, USA.

The pathogenic parasite Toxoplasma gondii invades host cells as part of its life cycle. Forward motion of the parasite during invasion is driven by rearward motion of adhesion receptors through the parasitic plasma membrane. These adhesion receptors are coupled to a unique actin (TgACT1) that forms very short filaments (~100 nm) for which there is no structure to lend them polarity. These are driven rearward by a fast, single-headed, class XIV myosin (MyoA) that is attached to an inner membrane complex. It is unknown how this system of un-oriented, short actin filaments and membrane-associated myosin can result in directional motility. Here we show that the motile apparatus of Toxoplasma is not pre-organized for directional motion, but rather becomes directional after a period of randomly oriented force generation. A laser trap was used to position microspheres on live Toxoplasma and to measure the transduction of force from TgACT1 and MyoA through cell surface adhesion receptors. We found that a ~50 second period of randomly oriented bead movement was followed by the force becoming oriented toward the rear (basal end) of the cell. The stall force was only 5.6 pN, and we see occasional series of 5 nm steps that may represent the activity of single MyoA. Force becomes directional at the basal end of the cell approximately 3 seconds later than at the apical end of the cell, but the magnitude of force generation was independent of location on the parasite. Addition of the actin filament stabilizer jasplakinolide abrogated directionality. These data suggest that MyoA is activated in Toxoplasma soon after receptor ligation, but that actin filament dynamics are critical to direction finding, and consequently to the regulation of host cell invasion.

3969-Pos Board B697

Probing Forces on Newly Generated Spindle Microtubule Minus-Ends

Christina L. Hueschen, Mary W. Elting, Dylan B. Udy, Sophie Dumont.
UCSF, San Francisco, CA, USA.

The mitotic spindle is a dynamic self-organizing machine that coordinates cell division and preserves genomic stability. The ability to focus microtubule minus-ends into poles is crucial to spindle structure and function. However, our understanding of pole-focusing forces has been limited by the challenges of labeling and imaging microtubule minus-ends in established spindles. Here, we used laser ablation to sever kinetochore-fiber microtubules in mammalian cells and probe how the cell detects and organizes newly generated microtubule minus-ends. Within a few seconds of ablation, the cell recognizes new minus-ends and begins pulling them poleward. These pole-focusing forces exist throughout metaphase and anaphase and can move chromosomes rapidly, dominating other spindle forces. Opposing forces on chromosomes from the other half-spindle are able to slow, though not stop, the pole-focusing response, as indicated by faster pole-focusing speeds in monopolar spindles and during anaphase than in metaphase bipolar spindles. Together, our data indicate that microtubule minus-end focusing forces operate broadly and rapidly and are of similar magnitude to other spindle forces. These pole-focusing forces are thus well-suited to robustly maintain spindle structural integrity despite rapid turnover of spindle components and mechanical challenges.

3970-Pos Board B698

Changes in Mechanical Properties of Actin Filaments of Astrocytes After Invasion by Trypanosoma Cruzi

Gerson F. Cote-Flórez, Juan Camilo Vargas-Zambrano, John Mario González, Manu Forero-Shelton.
Universidad de los Andes, Bogota, Colombia.

Astrocytes are the most abundant glia in the central nervous system and are responsible for neuronal protection and ion homeostasis, among others functions. Given its importance several atomic force microscopy studies have analyzed their topography and rigidity and found that actin filaments can be revealed by rigidity maps. Likewise, some studies found that the Young's modulus (modulus of elasticity) can decrease in astrocytes in the presence of drugs, especially actin synthesis inhibitors. However, no studies have reported how the stiffness of these cells can change during parasite invasion. In the present work, we determine the changes in the Young's modulus of astrocytes after infection by Trypanosoma cruzi using Atomic Force Microscopy. T. cruzi is a highly infective parasite that is responsible for Chagas disease, considered a neglected tropical disease, which can compromise the central nervous system during acute onset or in immunosuppressed individuals. In particular, we analyzed the topography and elasticity maps of the astrocytes, before and after infection. We find that the parasitic invasion significantly decreases cell and filament stiffness.

3971-Pos Board B699

Single Cell Measurements of Intracellular Signalling, and Motility, in Macrophage Cells Sensing a Bacterial Infection

Eugenia Cammarota[1], Jiro Sakai[2], Clare Bryant[2], Pietro Cicuta[1].
[1]Cavendish Laboratory, University of Cambridge, Cambridge, United Kingdom, [2]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom.

Macrophages are cells of the innate immune system in vertebrates. They are a cell type able to eat colloidal scale particles. In particular, these cells take up pathogenic bacteria, contributing to controlling a bacterial infection, and also acting to alarm the immune system and begin an inflammatory response.
Macrophages are motile, move around tissues. When the presence of pathogens is detected, a complex network of signal pathways is triggered; in this particular state the macrophage is said to be "activated". The aim of our research is the characterization of the activation process that takes place in macrophages on sensing the Salmonella bacteria. Working with cell culture systems, and exposing cells both to living bacteria, and to components of the bacteria, we investigate both the intracellular signalling, which feeds from a membrane receptor into the NF-kB signalling pathway, and also the phenotypic changes in cell motility and morphology.
Regarding the intracellular signalling, we make use of cell lines in which two key components are fluorescently labelled, and through a robust image segmentation routine we detect NF-kB translocation within the cell (repeated cycles of cytoplasm to nucleus), at single cell level. This allows us to quantitatively measure the intracellular variability, and to identify commonalities and differences within the clonal population.
Cell motility and shape are also affected by the detection of bacteria in the system: we quantify the migratory behaviour of macrophages, and how it changes depending on the different activation agents, linking the observations to putative biological functions. Experiments have been carried out observing cells behaviour after stimulation with Salmonella LPS (LPS is a molecule present on the outer membrane of gram-negative bacteria) and Interferon-γ (IFN-γ is a cytokine, expressed by macrophages as an intercellular signal).

3972-Pos Board B700

Traction Stress Dynamics During Chemotactic Amoeboid Cell Migration

Effie Bastounis, Reudi Meili, Begoña Álvarez-González, Juan Carlos del Álamo, Juan Lasheras, Richard Firtel.
UCSD, La Jolla, CA, USA.

Chemotaxis is involved in a broad range of biological phenomena such as during cancer metastasis. It requires a tightly regulated, spatiotemporal coordination of underlying biochemical processes that impact the mechanics of cell migration. In response to intrinsic and environmental cues, motile cells can adapt their migration effectively. Yet both the mechanisms by which this adaptation occurs and the role of the interactions between biochemistry and mechanics of cell migration are largely unknown.